FT-B8E000

SARS-COV-2 Nucleocapsid Protein IgG Antibody ELISA Kit

Catalog Number: RK04139

This ELISA kit used for quantitative determination of 2019-nCoV Nucleocapsid Protein IgG Antibody concentrations in serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Introduction

The kit applies for detecting the level of anti-SARS-CoV-2 (2019-nCoV) Nucleocapsid Protein IgG antibodies in serum and plasma.

Principle Of The Assay

This assay employs the Indirect immunoassay technique. A Nucleocapsid Protein specific for Nucleocapsid Protein Antibody has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any Nucleocapsid Protein Antibody present is bound by the immobilized protein. Following incubation unbound samples are removed during a wash step, and then a secondary antibody is added to the wells and binds to the combination of capture protein-Nucleocapsid Protein Antibody in sample. Following a wash to remove any unbound combination, a substrate is added. A colored product TMB is formed in proportion to the amount of Nucleocapsid Protein Antibody present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven Nucleocapsid Protein Antibody standard dilutions and Nucleocapsid Protein Antibody sample concentration determined.

Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Antibody Coated Plate	8×12	RM17517	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C.Reseal along entire edge of zip-seal.
Control Antibody(4000×)	1x20μL	RM17519	May be stored for up to 1 month at -20°C.*
Concentrated Secondary Antibody (500×)	1 ×30uL	RM17518	May be stored for up to 1 month at 2-8 °C.
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate Antibody Diluent (R2)	1 ×12mL	RM00024	
Wash Buffer(20x)	1 × 30mL	RM00026	
TMB Substrate	1 ×12 mL	RM00027	
Stop Solution	1 ×6 mL	RM00028	
Plate Sealers	4 strips		
Specification	1		

Sample Collection And Storage

1. Serum:

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

2. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles..

3. Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

4. Dilution:

Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.

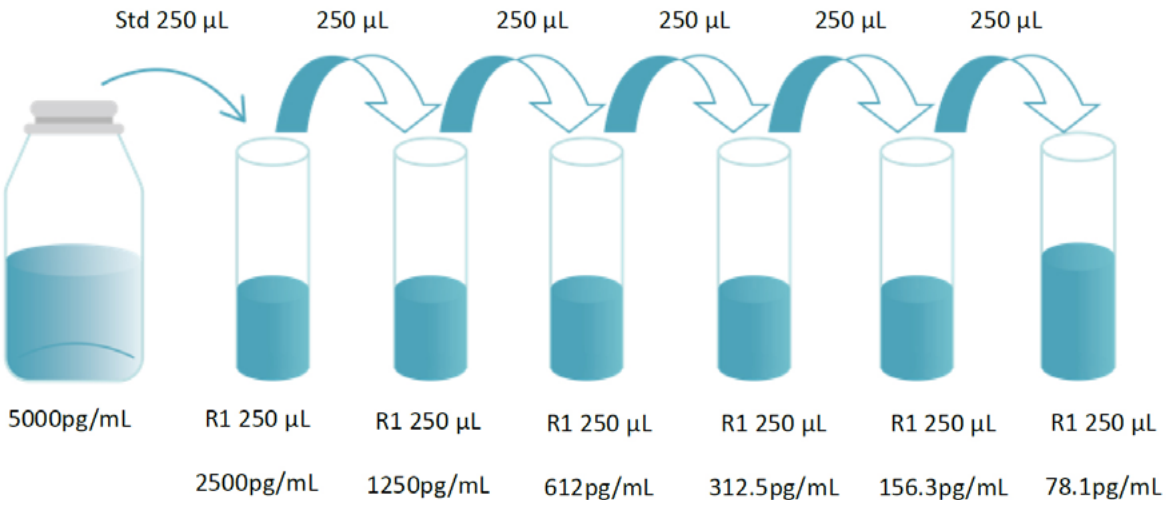
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

Experiment Materials

1. Microplate reader(measuring absorbance at 450nm, with the correction wavelength set at 570nm or 630nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**
2. **Control Antibody:** Dilute 1:4000 with the Control/Sample Diluent (R1) , sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (5000pg/mL), Prepare EP tubes containing Control/Sample Diluent (R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 5000, 2500, 1250, 625, 312.5, 156.3, 78.1, 0pg/mL). Redissolved solution (5000pg/mL), aliquot and store at -20°C— -70°C.



3. **Concentrated Secondary Antibody (500x):** Dilute 1:500 with the Secondary Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Secondary Antibody (500x)	Secondary Antibody Diluent (R2)
2	4μL	1996μL
4	8μL	3992μL
6	12μL	5988μL
8	16μL	7984μL
10	20μL	9980μL
12	25μL	12475μL

4. **Wash buffer:** Dilute 1:20 with double distilled or deionized water before use.

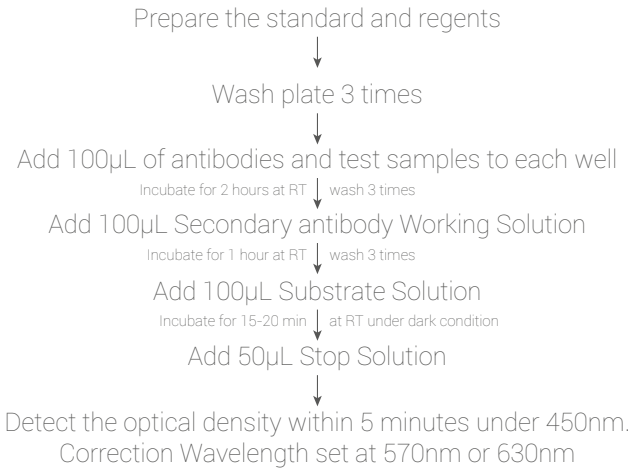
Wash Method

Aspirate each well and wash, repeating the process two times for a total of three washes.Wash by filling each well with **Wash Buffer** (300µL) using a squirt bottle,manifold dispenser,or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash,remove any remaining **Wash Buffer** by aspirating or decanting.Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100µL Control/Sample Diluent (R1) in blank well.
4. Add 100µL different concentration of Control Antibody and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Secondary Antibody (500X) Working Solution 15 minutes early before use.
7. Add Secondary antibody Working Solution in each wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at room temperature.
8. Warm-up the Microplate reader.
9. Repeat the aspiration/wash as in step 2.
10. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at room temperature .Protect from light.
11. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450nm. If wavelength correction is available, set to 570nm or 630nm. If wavelength correction is not available, subtract readings at 570nm or 630nm from the readings at 450nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450nm without correction may be higher and less accurate.

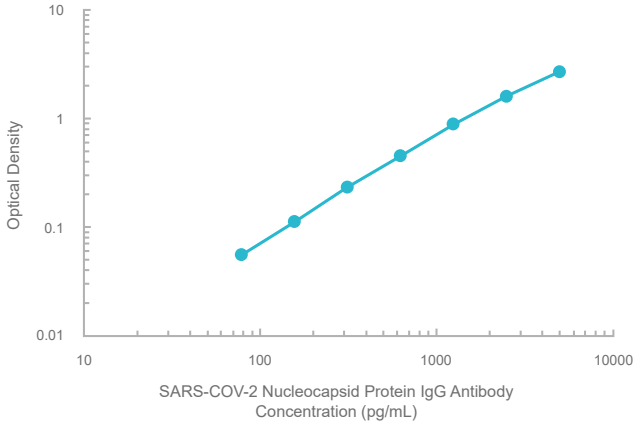
Assay Procedure Summary



Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the Nucleocapsid Protein IgG Antibody concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor..

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of Nucleocapsid Protein IgG Antibody assayed.

Sensitivity

The minimum detectable dose (MDD) of Nucleocapsid Protein IgG Antibody is typically less than 32.01pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.
This assay recognizes both recombinant and natural Nucleocapsid Protein IgG Antibody.

Precision

Inter-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	1035	3020	4980
Standard Deviation (SD)	42.4	123.8	229.1
Variable Coefficient CV (%)	4.1	4.1	4.6

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	1017	3005	5010
Standard Deviation (SD)	72.2	207.3	320.6
Variable Coefficient CV (%)	7.1	6.9	6.4

Recovery

Spike 3 different concentration of Nucleocapsid Protein IgG Antibody into healthy serum , calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	99	91-107
Plasma	100	87-113

Linearity

Spike high concentration of Nucleocapsid Protein IgG Antibody into 4 healthy serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	95	86-104
1:4	101	92-109
1:8	101	97-105
1:16	102	94-109